(b) a cellular targeting signal peptide not normally associated with the catalytic domain selected to target the GlcNAc transferase II enzyme to the ER or Golgi apparatus of the host cell;

whereby, upon passage of the recombinant glycoprotein through the ER or Golgi apparatus of the host cell, a recombinant glycoprotein comprising a $GlcNAc_2Man_3GlcNAc_2$ glycoform is produced.

REMARKS

Applicant acknowledges with gratitude the Examiner's entry of their 3/7/05 amendment. The claim amendments submitted herewith, as discussed below, are based on the claims submitted with that amendment.

## I. Claim Amendments

Claims 35, 39, 40, 42-50, 52-54, 57-73 and 79-80 are currently pending. Claims 1-34, 36-38, 41-43, 51 and 54-56 have been canceled previously, and claims 74-78 are non-entered. With this response, claim 57 has been canceled, and claims 35, 67, 70, 71, 79 and 80 have been amended to define more clearly what applicant considers to be his invention. Each of these amendments is supported by the specification as originally filed and none adds new matter. The amendments are addressed below in the context of addressing the Examiner's outstanding rejections.

### 35 U.S.C. § 112 Claim Rejections – Enablement

Claims 35, 39, 40, 42-50, 52-54 and 57-73, 79 and 80 stand rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement in the application as originally filed. Claim 57 is currently canceled, rendering this rejection moot as to that claim.

The Examiner points to applicant's post-filing date publication, Choi et al., for teaching that "proper combination of the catalytic domain and leader sequence is important to practice the claimed method." (Action p. 3) This teaching is also found in the instant application as originally filed. See, e.g., page 26, line 26 – page 27, line 18, which describes a preferred embodiment of the invention involving the selection of desired glycosylation phenotypes after a host cell of choice is transformed one or more times with a library of catalytic domain and targeting sequence fusion constructs:

> A library including at least two genes encoding exogeneous (*sic*) glycosylation enzymes is transformed into the host organism, producing a genetically mixed population. Transformants having the desired glycosylation phenotypes are then selected from the mixed population.... In an especially preferred embodiment, the DNA library includes genetic constructs encoding fusions of glycosylation enzymes with targeting sequences for various cellular loci involved in glycosylation especially the ER, cis Golgi, medial Golgi, or trans Golgi.... Selection of desired phenotypes may be performed after each round of transformation or alternatively after several transformations have occurred.

It is clear from the above quotation that selection of a desired glycosylation phenotype is based on enzymatic activity produced *in the chosen host cell* by *the combination* of catalytic domain and targeting sequence and depends on how and where the glycosylation enzyme is localized and expressed in the host cell. One of the advantages of the present invention is that, after transformation, selection and N-glycan screening, the host cell itself indicates whether an encoded glycosylation enzyme will result in a desired phenotype in the host cell of choice, such as one capable of producing "in excess of 30% $Man_5GlcNAc_2$" as recited in claim 35. See also, e.g., page 30, lines 4-14 and page 31, lines 8-11; original claims 14, 24 and 29.

The original application teaches the skilled worker that a given enzymatic catalytic domain will function more efficiently when targeted to the ER or Golgi apparatus of a particular host cell if that catalytic domain has a pH optimum relatively close to (i.e., within a 1.4 pH unit range) the average pH optima of other glycosylation enzymes already present in the ER or Golgi of the host cell. Applicant agrees with the Examiner (Action, page 3) that the "specification teaches that most active enzymes in ER and golgi have pH [optima] between 6.5-7.5. As such, 1.4 units within average pH will be in the range of 5.6-8.4." This does not mean, however, that *any or all* catalytic domains having activities within that pH range will function optimally. That will depend on which cellular targeting sequence is used and how the fusion protein localizes and/or is expressed in the host cell. Rather, the instant application teaches the skilled worker the importance of the pH optima of glycosylation catalytic domains. The prior art, in contrast, used a mannosidase catalytic domain that had a pH optimum of 5.0, and thus would be expected from the beginning not to function well at the particular location to which it was targeted (average pH of 7.0) -- no matter what the fusion. The application teaches that catalytic domains be selected based on pH optima and then be used i*n combination with* one or more cellular targeting sequences to identify (by expression and screening in the host) a glycosylation enzyme having optimal activity in the ER or Golgi apparatus of a chosen lower eukaryotic host cell.

Applicant has amended the claims to recite this distinction with more particularity. Specifically, claims 35, 67, 70, 71, 79 and 80 have each been amended to recite that the nucleic acid transformed into the host cell encodes a fusion enzyme comprising a catalytic domain having a particular pH optimum range (5.1 to 8.0) fused to a cellular targeting sequence that targets the catalytic domain to the ER or Golgo; and that the fusion enzyme is selected to function optimally at

the location in the host cell (ER or Golgi) to which the enyzme is targeted by means of the targeting sequence. Specifically, the claims have been amended to recite that the nucleic acid transformed into the host cell encodes a glycosylation enzyme that is:

> selected to have optimal activity in the ER or Golgi of said host cell, the enzyme comprising: (a) a [...] catalytic domain having optimal activity in said ER or Golgi at a pH between 5.1 and 8.0; fused to (b) a cellular targeting signal peptide not normally associated with the catalytic domain selected to target the [...] enzyme to the ER or Golgi apparatus of the host cell.

Original support for these amendments is found, e.g., at page 26, line 26 – page 27, line 18; see also, e.g., page 30, lines 4-14 and page 31, lines 8-11; and original claims 14, 24 and 29, as described above. Original support in the application for the pH range of 5.1 to 8.0 is found for alpha-1,2 mannosidases, e.g., at page 12, line 21 and page 25, lines 1-2; for mannosidases in general in original claim 7; and for GnT's and other transferase enzymes, e.g., in original claim 16.

In addition, the Examiner points to a hybrid *A. nidulans* mannosidase enzyme disclosed in Choi et al. which has a pH optimum of 6 and "generally results in low yield of the desired N-glycan structure" (citing Choi et al., page 5026, 1st col., 3d para., last two lines.) Thus, according to the Examiner, this disclosed *A. nidulans* mannosidase would fall within applicant's claims but would not be expected to produce the recited "in excess of 30 mole %" of the desired N-glycan structure. Applicant respectfully traverses this objection.

As explained above, the application does not teach nor do the claims provide that *any and all* glycosylation enzymes will produce the claimed N-glycan structures in desired amounts if they have "a pH optimum within 1.4 pH units of the average pH optimum of glycosylation-related enzymes in the subcellular location where the domain is targeted" (as recited in the former claims,

now amended). Rather, it is the *combination* of a catalytic domain having an appropriate pH optimum with a cellular targeting sequence – and how that fusion is expressed and localized in the particular host cell of choice – that determines whether the catalytic domain will function efficiently in the subcellular location it is targeted to. Thus, the same *A. nidulans* catalytic domain might work efficiently in a different lower eukaryotic host cell and/or in a different cellular targeting sequence fusion construct.

It is well-established that not every embodiment that falls within a claim need be operable for a claim to be enabled. See MPEP 2164.08(b): "The presence of inoperative embodiments within the scope of a claim does not necessarily render a claim nonenabled.... Although, typically, inoperative embodiments are excluded by language in a claim (e.g., a preamble), the scope of the claim may still not be enabled where undue experimentation is involved in determining those embodiments that are operable." That is not the case here. One of skill in the art can use methods disclosed in the instant application to engineer and to screen lower eukaryotic host cells for those with desired glycosylation phenotypes without undue experimentation.[1] The skilled worker knows how to identify such host cells using methods well known in the art and disclosed in the application as filed (see, e.g., page 31, line 8 – page 32, line 9).

Nonetheless, applicant's claims as amended are no longer subject to this criticism. As amended, the claims recite more clearly that it is the catalytic domain that is selected to have a particular pH optimum "between 5.1 and 8.0"; and that the activity of that domain in combination

[1] In applicant's experience, it is typical to find about one in four lower eukaryotic host cells produced according to the methods of the invention that produce desirable glycosylation phenotypes in a lower eukaryotic host cell of choice.

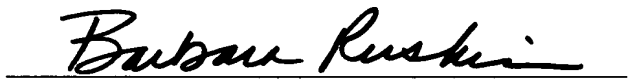
with the cellular targeting sequence in the particular host cell is selected for on the basis of producing a desired glycosylation phenotype (such as the recited N-glycans) in the host cell. These claim limitations act to exclude further certain inoperative embodiments from the claims, such as the *A. nidulans* example raised by the Examiner.

Based on the above amendments, applicant respectfully requests that the Examiner withdraw the 112 rejections based on lack of enablement and that the amended claims be favorably considered and allowed.

## II. Conclusion

Entry of this Amendment and allowance of the claims as submitted herewith is respectfully requested. Applicant's undersigned representative requests a telephonic interview with the Examiner if it would expedite place the amended claims in condition for allowance.

Respectfully submitted,



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